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DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH SOME COMMON MEDICINAL PLANTS OF LAMIACEAE FROM ANAIMALAI HILLS

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ABSTRACT

The present study is aimed to list out some common medicinal plants of Lamiaceae and to determine the Arbuscular Mycorrhizal Fungi spore population and its colonization in twenty common medicinal plants of Lamiaceae in rainy season (July, August and September) of Anaimalai Hills. The plants were arranged alphabetically with their botanical names, the common name, parts used and its medicinal uses. Majority belong to herbaceous category. In this study, all the twenty plants were found to have AMF association. Present study revealed that AMF colonization and spore population gradually increased in rainy season. Maximum AMF colonization and spore population was noticed in *Plectranthus amboinicus* in September month. Minimum AMF colonization in *Scutellaria colebrookiana* and *Ocimum tenuiflorum* showed minimum spore population in July month. Totally 40 spores were isolated from the rhizosphere soils of common medicinal plants of Lamiaceae. *Glomus* was recorded as dominant AMF species.

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INTRODUCTION

The earth is home to a rich and diverse array of living organisms, whose genetic diversity and relationships with one another and with their physical environment constitutes biodiversity. One such area with rich biodiversity which runs majestically parallel to the west coast of India, the Western Ghats are sprawling. Medicinal plant species of Western Ghats represent a variety of life form ranging from lichens to perennials. Among the various medicinal plants, the plants belonging to the family Lamiaceae are probably the most popular.

Lamiaceae are important and many are of great economic importance for their pharmaceutical properties ^[1]. They are widely used in home remedies and horticulture. Plants of Lamiaceae contain a large number of polyphenolic compounds ^[2]. These compounds are involved in a wide range of physiological and ecological activities providing resistance to bacterial, fungal and viral infections ^[1]. Hence, commercial cultivation of medicinal plants has started.

Healthy, fertile soils are characterised by diverse population of micro- organisms. Arbuscular Mycorrhizal Fungi (AMF) are the most common type, constitute a major group of soil microbial community ^[3]. In recent years, AMF gained significant importance because of their potential to improve growth and productivity of the plants by increasing the nutrient uptake ^[4]. AMF colonization varies with season and its effects also influence the establishment of plants under field condition.

The increased interest in AM fungi has prompted numerous surveys, aimed at enumerating and assessing AM fungi in a particular habitat and plant species ^[5]. Surprisingly, very little information on AM association in temperate soils is available. Medicinal plants, particularly aromatic plants in India were originally reported to be non-mycorrhizal probably due to the presence of secondary metabolites ^[6]. Later, many workers found the roots of various medicinal plants to be mycorrhizal ^[7,8,9,10,11,12]. Therefore, the present study is aimed to list out some common medicinal plants of Lamiaceae and to determine the AMF spore population in rhizosphere soils and its colonization in twenty medicinal plants of Lamiaceae in rainy seasons of Anaimalai Hills.

MATERIALS AND METHODS

The Western Ghats, a valuable repository for biodiversity after the Himalayas, is one of the 25 mega diversity hot spots of the world ^[13]. The Anaimalai Hills is a range of mountains in the Western Ghats in TamilNadu state of India. The name 'Anai' means elephant and 'Malai' means Hill, thus Elephant Hill. These Hills are located between 10°13' and 10°31'N and 76°52' and 77°23'E. These hills have been recognized as one of the eight hottest hotspots of biodiversity ^[13].

Collection of medicinal plants

A field survey was made in the Anaimalai hills. Twenty common medicinal plants were selected for collection. The list of all the plant species included in the study was prepared with the help of the published literatures ^[14,15,16,17]. Medicinal uses of these plant species have been collected from various reference books and research papers ^[9,18,19,20,21].

Sampling

The root and rhizosphere soil samples of twenty common medicinal plants belonging to the family Lamiaceae were randomly collected from Anaimalai Hills and screened for association of AM fungi. Three replicates of each plant were randomly sampled i.e. three plants were dug out, placed in polythene bags and transported to the laboratory. Root samples were freshly processed, whereas the rhizosphere soil samples were stored at 4°C until they were processed.

AMF root colonization

Root colonization was observed by rapid clearing and staining technique ^[22]. For AM colonization assessment, root samples were cleared with 10% KOH, acidified with 1N HCL and stained in lactoglycerol trypan blue (0.05%). Quantification of AM fungal colonization was carried out using the grid- line intersection method and expressed as percentage root colonized ^[23]. The stained roots were mounted on microscopic slides and the segments were examined by light microscopy. Percent root colonization was determined using formula as mentioned under:

$$\% \text{ Root Colonization} = (\text{Number of positive segments} / \text{Number of segments observed}) \times 100.$$

AM Fungi spore population

AM fungal spores were isolated from 10g rhizosphere soil samples using wet sieving and decanting method ^[24]. For this, 10g of soil was mixed with 100ml of water and the supernatant was decanted through stacked sieves. Debris on the sieve was collected in beaker. Spores were recovered on filter paper and quantification of spores was carried out under microscope. Diagnostic slides with spores/Sporocarps were prepared using polyvinyl alcohol lactoglycerol (PVLG) as mountant. Both broken and unbroken spores were examined under compound microscope.

Identification of AM fungi

Spores were examined and identified according to spore morphology and wall characters using manuals ^[25,26,27,28].

RESULTS

The list of twenty medicinal plants and their parts used as medicine collected from Anaimalai Hills are given in Table- 1. The names of the plants are arranged alphabetically with their botanical names, the common name, parts used and its medicinal uses. These plants are well distributed and the majority belonging to the herbaceous category.

Table 1. List of medicinal plants (Lamiaceae), their common name, part(s) used and its medicinal uses.

S.No	Name of the plant	Common name	Parts used	Medicinal uses
1	<i>Anisomeles malabarica</i> R. Br. ex Sims	Malabar catmint	Leaves	It is used to cure rheumatism, intermittent fever, and stomach infection. It is also used against scorpion sting and snakebite.
2	<i>Anisomeles indica</i> Kuntze (L.) R. Br.	Indian catmint	Whole plant	Decoction of aerial part is taken orally in burning sensation during urination.
3	<i>Hyptis suaveolens</i> (L.) Poit.	American mint	Whole plant	Leaves are used to cure parasitic cutaneous disease. Crude leaves are used in colic and stomachache. Seeds are used in boils, burns, wounds and various skin diseases.
4	<i>Leucas aspera</i> (Willd.) Link.	Common leucas	Leaves	It is used for cough, cold, painful swellings and chronic skin eruptions. It is also used in snake bite and to cure chicken pox.
5	<i>Leucas vestita</i> Benth.	Leucas	Leaves	It is used in scabies, skin diseases, cold, cough, and headache and also against snakebite.
6	<i>Mentha arvensis</i> Linn.	Japanese mint	Leaves	Used to treat liver and spleen diseases, asthma and jaundice, stomach disorders and headache, In indigestion and rheumatic pains.
7	<i>Mentha piperita</i> Linn.	Peper mint	Leaves	It is administered in serious nervous disorders, in headache, vaginal discharges with nervous symptoms.
8	<i>Ocimum gratissimum</i> L.	Shrubby basil/ clocimum	Leaves	It is used in respiratory infection, diarrhoea, headache, fever, skin diseases and pneumonia.
9	<i>Ocimum basilicum</i> Linn.	Sweet basil	Roots, leaves, flowers and seeds	They are used in heart and brain diseases, chronic pain in the joints, asthma, inflammations, toothache, headaches and cure ring worm.
10	<i>Ocimum tenuiflorum</i> Linn.	Holy basil	Leaves, seeds and roots	They are used in malarial fevers, gastric disorders and in hepatic infections. It is also used in nerve disorders and to increase memory power.
11	<i>Origanum vulgare</i> L.	Oregano	Flower and leaves	They are used in whooping cough, gastrointestinal disorders, respiratory disorders, dental caries, rheumatoid arthritis and urinary tract disorders.
12	<i>Origanum marjorana</i> L.	Sweet marjorana	Whole plant	Used for sprains, bruises stiff and paralytic limbs, tooth ache and for hot fomentation in acute diarrhoea.
13	<i>Orthosiphon diffuses</i> Benth.	Java tea	Leaves	Used in urinary infections, fever, influenza, hepatitis, diabetes, rheumatism, tonsillitis, menstrual disorders and hypertension.
14	<i>Plectranthus amboinicus</i> (Lour.) Spreng.	Indian borage	Leaves	Used in cough, cold and fever. Cure epilepsy, convulsion, kidney and bladder stones. For urinary diseases and vaginal discharges.
15	<i>Plectranthus malabarica</i> (Benth.) R.H. Willemse.	Cockspur flower	Leaves	Used in cold, cough, to reduce body pain, wounds, to stimulate hair growth and also in heart diseases.
16	<i>Plectranthus mollis</i> (Aiton) Spreng.	Spur weed	Leaves	It is used for stopping bleeding and as mosquito repellent. Used in cuts and wounds, boils.
17	<i>Pogostemon patchouli</i> Pellet	patchouli	Leaves	Used in treatment of nausea, diarrhoea, cold, cough, fever, headache and antidote for snake bite.
18	<i>Salvia officinalis</i> Linn.	Sage	Leaves	Used in inflammation of the mouth and throat, in the treatment of cancer. In throat infection and skin diseases.
19	<i>Scutellaria colebrookiana</i> Wall.	Skull caps	Whole plant	It is used against cuts and wounds.
20	<i>Thymus vulgaris</i> Linn.	Common thyme	Whole plant	Used in bronchitis, laryngitis, chronic gastritis, dyspepsia, tonsillitis and laryngitis.

Diversity of native AMF

All the twenty common medicinal plant species of the family Lamiaceae studied, exhibited AM fungal association. AMF colonization in roots and the spore population in the rhizosphere soil samples of all the species studied showed wide range of variation. Root colonization Percentage and mycorrhizal spore counts steadily increased in rainy season

AMF root colonization

The maximum infection (75.33%) was recorded in *Plectranthus amboinicus* in September month, whereas minimum infection (11.66%) in *Scutellaria colebrookiana* was observed in the month of July (Table- 2). The root colonization was observed to vary from 11.67% to 64.33%, 16.67% to 70% and 21% to 75.33% during the months of July, August and September respectively (Table- 2).

Table 2: Root colonization of AM Fungi in common medicinal plants of Lamiaceae.

S.NO	Name of the plant	% Root colonization		
		July	August	September
1	<i>Anisomeles malabarica</i>	20.67± 2.49	25.33± 4.11	35± 3.27
2	<i>Anisomeles indica</i>	14.33± 3.29	17.67± 2.05	24.33± 3.68
3	<i>Hyptis suaveolens</i>	18± 2.94	22.33± 2.05	26.33± 3.86
4	<i>Leucas aspera</i>	22.67± 2.49	32.33± 2.62	34.33± 3.29
5	<i>Leucas vestita</i>	14.67± 2.05	21± 2.16	24.67± 3.68
6	<i>Menthe arvensis</i>	32.67± 2.49	33± 2.45	35.33± 4.11
7	<i>Mentha piperita</i>	17± 2.45	19.67± 1.25	26.33± 3.86
8	<i>Ocimum gratissimum</i>	13.33± 2.49	18.67± 1.25	26.33± 3.29
9	<i>Ocimum basilicum</i>	21± 2.45	29.67± 2.05	32.33± 2.05
10	<i>Ocimum tenuiflorum</i>	12.33± 2.05	17.33± 2.05	19± 3.27
11	<i>Origanum vulgare</i>	44.33± 3.29	50± 1.63	57.67± 2.05
12	<i>Origanum marjorana</i>	30± 0.82	37± 2.45	42.67± 2.49
13	<i>Orthosiphon diffuses</i>	20± 1.63	26± 3.27	31± 3.27
14	<i>Plectranthus amboinicus</i>	64.33± 3.29	70± 1.63	75.33± 3.68
15	<i>Plectranthus malabarica</i>	29.67± 2.05	33.67± 2.87	38.33± 1.69
16	<i>Plectranthus mollis</i>	32.33± 2.05	38± 1.63	42± 2.16
17	<i>Pogostemon patchouli</i>	24.67± 2.87	30.33± 2.05	36± 4.32
18	<i>Salvia officinalis</i>	57.33± 2.05	62± 2.16	69.33± 2.49
19	<i>Scutellaria colebrookiana</i>	11.67± 1.69	16.67± 2.49	21± 2.94
20	<i>Thymus vulgaris</i>	39.33± 1.69	47.67± 2.05	52.67± 2.05

AM Fungal spore population

The spore population was minimum in July, gradually increased in August and was maximum in September. The spore population was observed to vary from 155 to 511 spores, 165 to 542 spores and 186 to 595 spores during July, August and September months respectively. The maximum number of spores (595) was observed in *Plectranthus amboinicus* and followed by *Origanum vulgare* (525) in September month. The minimum number of spores (155) was observed in *Ocimum tenuiflorum* during July (Table- 3).

Table 3: AM Fungal spore population in common medicinal plants of Lamiaceae.

S.NO	Name of the plant	Total No. of spores/100g of soil		
		July	August	September
1	<i>Anisomeles malabarica</i>	251± 5.31	275± 4.11	296± 3.74
2	<i>Anisomeles indica</i>	159± 6.79	184± 4.32	206± 2.87
3	<i>Hyptis suaveolens</i>	255± 4.11	296± 4.19	316± 3.74
4	<i>Leucas aspera</i>	313± 2.49	345± 4.08	396± 3.86
5	<i>Leucas vestita</i>	213± 2.45	244± 4.32	296± 3.29
6	<i>Menthe arvensis</i>	324± 3.27	355± 4.11	378± 1.69
7	<i>Mentha piperita</i>	267± 4.19	285± 4.09	315± 3.68
8	<i>Ocimum gratissimum</i>	194± 7.12	219± 6.59	265± 3.68
9	<i>Ocimum basilicum</i>	284± 6.02	315± 4.09	375± 3.68
10	<i>Ocimum tenuiflorum</i>	155± 6.94	165± 3.68	186± 4.49
11	<i>Origanum vulgare</i>	442± 4.32	495± 4.11	525± 4.11
12	<i>Origanum marjorana</i>	181± 2.49	206± 4.32	256± 3.29
13	<i>Orthosiphon diffuses</i>	511± 7.76	544± 4.78	595± 4.11
14	<i>Plectranthus amboinicus</i>	355± 4.12	385± 4.08	424± 4.19
15	<i>Plectranthus malabarica</i>	346± 4.32	396± 3.29	434± 4.49
16	<i>Plectranthus mollis</i>	319± 2.62	363± 2.94	377± 3.09
17	<i>Pogostemon patchouli</i>	347± 2.05	378± 4.03	415± 4.11
18	<i>Salvia officinalis</i>	387± 2.05	415± 3.68	446± 3.29
19	<i>Scutellaria colebrookiana</i>	245± 3.68	263± 3.09	296± 3.29
20	<i>Thymus vulgaris</i>	208± 5.56	258± 4.64	286± 3.86

Totally, 40 AMF spores were isolated from the rhizosphere soil samples of medicinal plants of the family Lamiaceae and were identified (Table- 4). These 40 spores come under six genera namely *Acaulospora*, *Archeospora*, *Entrophospora*, *Gigaspora*, *Glomus*, and *Scutellospora*. Arbuscular Mycorrhizal Fungi belonging to genus *Glomus* were dominant when compared to all other genus.

Table 4. List of spores isolated from the root zone soil of medicinal plant species of family Lamiaceae.

S. No	AM fungal species	Authorities
I	<i>Acaulospora</i>	
1	<i>A. bireticulata</i>	F.M. Rothwell & Trappe
2	<i>A. delicata</i>	C. Walker, C.M. Pfeiff. & Bloss
3	<i>A. denticulata</i>	Sieverd. & S. Toro
4	<i>A. morrowae</i>	Spain & N.C. Schenck
5	<i>A. scrobiculata</i>	Trappe
II	<i>Archaeospora</i>	
6	<i>A. trappei</i>	(R.N. Ames & Linderman) J.B. Morton & D. Redecker emend Spain
III	<i>Entrophospora</i>	
7	<i>E. infrequens</i>	(I.R. Hall) R.N. Ames & R.W. Schneid.
IV	<i>Gigaspora</i>	
8	<i>G. decipiens</i>	I.R. Hall & L.K. Abbott
9	<i>G. gigantean</i>	(T.H. Nicolson & Gerd.) Gerd. & Trappe
10	<i>G. margarita</i>	W.N. Becker & I.R. Hall.
V	<i>Glomus</i>	
11	<i>G. aggregatum</i>	N.C. Schenck & G.S. Sm.
12	<i>G. ambisporum</i>	G.S. Sm. & N.C. Schenck
13	<i>G. botryoides</i>	F.M. Rothwell & Victor
14	<i>G. citricola</i>	D.Z. Tang & M. Zang
15	<i>G. constrictum</i>	Trappe
16	<i>G. deserticola</i>	Trappe, Bloss & J.A. Menge
17	<i>G. dimorphicum</i>	Boyetchko & J.P. Tewari
18	<i>G. drummondii</i>	Blaszk. & C. Renker
19	<i>G. etunicatum</i>	W.N. Becker & Gerd.
20	<i>G. fasciculatum</i>	(Thaxt.) Gerd. & Trappe emend. C. Walker & Koske
21	<i>G. fuegianum</i>	(Speg.) Trappe & Gerd.
22	<i>G. fulvum</i>	(Berk. & Broome) Trappe & Gerd.
23	<i>G. geosporum</i>	(T.H. Nicolson & Gerd.) C. Walker
24	<i>G. hoi</i>	S.M. Berch & Trappe
25	<i>G. intraradices</i>	N.C. Schenck & G.S. Sm.
26	<i>G. macrocarpum</i>	Tul. & C. Tul.
27	<i>G. microcarpum</i>	Tul. & C. Tul.
28	<i>G. mosseae</i>	(T.H. Nicolson & Gerd.) Gerd. & Trappe
29	<i>G. radiatum</i>	(Thaxt.) Trappe & Gerd.
30	<i>G. reticulatum</i>	Bhattacharjee & Mukerji
31	<i>G. rubiformis</i>	(Gerd. & Trappe) R.T. Almeida & N.C. Schenck
32	<i>G. sinuosum</i>	R.T. Almeida & N.C. Schenck
33	<i>G. walkeri</i>	Blaszk. & C. Renker
VI	<i>Scutellospora</i>	
34	<i>S. calospora</i>	(T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders
35	<i>S. dipurpureus</i>	J.B. Morton & Koske
36	<i>S. gregaria</i>	(N. C. Schenck & T.H. Nicolson) C. Walker & F.E. Sanders
37	<i>S. heterogama</i>	(T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders
38	<i>S. nigra</i>	(J.F. Redhead) C. Walker & F.E. Sanders
39	<i>S. pellucid</i>	(T.H. Nicolson & N. C. Schenck) C. Walker & F.E. Sanders
40	<i>S. persica</i>	(Koske & C. Walker) C. Walker & F.E. Sanders

DISCUSSION

As mentioned in the introduction, the significance of AM fungi in medicinal crop production is widely appreciated and therefore the necessity of using AM fungi as a bioinoculant for improving crop production is increasingly realized especially in transplantable medicinal crops^[29,30]. However, the information available on the use of AM fungi in medicinal plants is limited in Tamil Nadu, India. Medicinal plants especially aromatic plants in India were originally reported to be non-mycorrhizal, probably due to the presence of various secondary metabolites^[6]. Later, many investigations revealed that the mycorrhizal fungi enhance the growth and productivity of aromatic plants. The occurrence and biodiversity of AM fungi in native common medicinal plants of Lamiaceae have not been described.

Diversity

Occurrence of AM fungi is well documented in a wide range of natural ecosystem. The climatic variation also influences the selection of AMF as it regulates the incidence of certain specific strains in the soil ^[31]. Among the climatic factors, relative humidity and rainfall influenced root colonization. The diffused oxygen to rhizosphere from the elevated soil water may reason for increasing AMF. This observation has been supported by many workers, who reported that the rainy season show higher root colonization and spore number. Least activity of AM fungi in other seasons than rainy season might be due to reduced translocation of carbohydrates towards root ^[32]. Hence, in the present study all the twenty common medicinal plants of Lamiaceae in Anaimalai Hills were collected in different months of rainy season to assess the AM association.

All the twenty plant species studied exhibited AM fungal association (Tables- 2 and 3). AM fungal colonization in roots and the spore population in the rhizosphere soil samples showed wide range of variation. The level of AM fungal association depends on root morphology, metabolism and rate of plant growth ^[33]. Per cent root colonization and mycorrhizal spore counts steadily increased in months of rainy season. The report of Ragupathy and Mahadevan (1993) also showed highest per cent root colonization during rainy season. Sangeeta Kaushal (2000) and Nisha, et al., (2010) also reported the maximum root colonization and spore population in rainy season.

The definition of Lindsey (1984) was kept in mind while describing a species as mycorrhizal. A species was considered mycorrhizal if roots contained one of the following combinations of AM fungal structures in the primary cortex, hyphae + arbuscules, hyphae + pelotons or hyphae + vesicles. To qualify as an AM species, the presence of arbuscules was considered critical ^[37]. Totally 40 AMF spores were isolated from the rhizosphere soil samples in plants of family Lamiaceae. 40 AMF spores were belonging to six genera (Table- 4). AMF belonging to genus *Glomus* were dominant and this was supported by the reports of Gautam and Roy (2009) and Nisha, et al., (2010).

This confirms that the plants are colonised by AM fungi. The root colonization and spore population was also found to be high in rainy season. It is also apparent that rainy season may be considered as the best season for AM fungi assessment and for the propagation of plants by the application of AMF as bioinoculants. These relations suggest that environmental factors strongly influence AM root colonization and sporulation.

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